

α -Tocopherol in plasma and milk from organically managed dairy cows fed natural or synthetic vitamin E or seaweed

HÅVARD STEINSHAMN¹, INGVILD STEINNES LUTEBERGET²,
SØREN K JENSEN³, ERLING THUEN²

¹ Bioforsk - Norwegian Institute for Agricultural and Environmental Research,
Organic Food and Farming Division, Norway, <http://www.bioforsk.no>,
eMail: havard.steinshamn@bioforsk.no

² Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Nor-
way, <http://www.umb.no>, eMail: erling.thuen@umb.no

³ Department of animal Sciences, Aarhus University, Denmark, <http://www.au.dk>, eMail:
sorenkrogh.jensen@agrsci.dk

Abstract

The objective was to compare the effects of supplementing lactating dairy cows with synthetic (All-rac), natural (RRR) all-rac- α -tocopheryl acetate or seaweed with a control on the concentration of α -tocopherol in blood and milk. Twenty four dairy cows in mid lactation, fed an organic feed ration, were randomly allocated to the four treatments in a replicated Latin square design. Plasma and milk α -tocopherol concentrations were higher in RRR and All-rac than in the other treatments and higher in RRR than in All-rac. RRR- α -tocopherol was the predominant stereoisomer (> 86%), in both plasma and milk, whereas the remaining part was largely made up by the three synthetic 2R isomers. In cows fed the control, seaweed and RRR, the proportion of RRR- α -tocopherol in plasma and milk constituted more than 97% of the total α -tocopherol. The study demonstrated that dairy cows in mid and late lactation have preferential uptake of RRR- α -tocopherol compared to other stereoisomers.

Key words: Vitamin E, stereoisomer, macro algae, cattle

Introduction

In Northern Europe, ruminants are fed preserved forages, mainly silage, for a long period during winter time. Forage preservation and storage may reduce the content of vitamin E substantially, and supplementation with vitamin E is recommended also in organic production as low plasma levels of vitamin E have been observed in organic managed sheep and cattle (Beckman et al. 2010, Govasmark et al., 2005). Natural vitamin supplement sources are generally difficult and expensive to derive, and there is currently derogation from the EU regulation on organic production to use synthetic vitamin A, D and E (European Commission 2008). However, there is an aim in organic production to avoid synthetic vitamins and it is therefore important to find alternative sources. It is also well known that the natural vitamin E isomer, RRR- α -tocopherol, has higher bioavailability than synthetic all-rac- α -tocopherol that contains equal amount of all eight isomers (Meglia et al. 2006). Seaweed has been used as supplement to ruminants in Norway for centuries, and recent research may indicate that supplementation with seaweed may up-regulate antioxidant responses in animals (Allen et al. 2001). The objective of this study was to compare the effects of supplementing lactating dairy cows with synthetic all-rac- α -tocopheryl acetate or natural RRR- α -tocopheryl acetate or with seaweed on the concentration of α -tocopherol and its stereoisomers in blood and milk.

Material and methodology

Twenty four Norwegian Red cattle in mid lactation (164 d in milk, SD=30) were used in a replicated 4 × 4 Latin square design with 28 d periods. The experiment was carried out in agreement with the laws and regulations controlling experiments on live animals in Norway. The cows were fed a basic ration of grass-red clover silage *ad libitum* and 3 kg/d flat rate of a concentrate mixture. The silage and the concentrate were produced according standards for organic production. The ration was supplemented with the following four experimental diets: SW, commercially available dried and ground macro algae *Ascophyllum nodosum* (200 g/d); RRR, natural vitamin E (2280 mg RRR- α -tocopherol/d); *All-rac*, synthetic vitamin E (2280 mg *all-rac*- α -tocopherol/d) and a Control without extra α -tocopherol or macro algae. The RRR, *All-rac* and Control supplements were given at a daily rate of 0.66 kg per cow and consisted of barley (0.75), molasses (0.04), minerals and vitamins A and D (0.21) and the vitamin E sources, whereas the SW was a mixture of macro algae (0.30), barley (0.52), molasses (0.04) and minerals (0.18) in order to be similar to the other treatments with respect to total mineral supplementation and applied at rate of 0.77 kg/d iso-energetically equal to the other treatments. Milk yield and feed intake was recorded and milk and blood samples were taken in the last week of each period. The content of α -tocopherol in feed, plasma and milk were analysed according to Jensen and Nielsen (1996). Data was analysed using the PROC MIXED procedure in SAS with treatment and period as fixed effects and replicate and cow within replicate as random effects. Differences among treatment means were detected using the Tukey procedure. Significance was declared at P< 0.05.

Results

The dietary treatments had no effect on silage intake (14.6 kg DM/d, P=0.90), total feed intake (18.1 kg DM/d, P=0.99) or milk yield (16.0 kg/d, P=0.39). The intake of α -tocopherol from the RRR and *All-rac* supplements was 75 % lower than planned (Table 1), mainly due to high temperature during the pelleting process that reduced the α -tocopherol content of the supplements by 50-66 %. Still, the total intake of α -tocopherol on RRR and *All-rac* diets was about 1.6 times higher than on the control and SW. Intake of the RRR- α -tocopherol isomer was about 70% higher on RRR than on *All-rac* and control, whilst the intake of the other 2R and 2S isomers was highest on the *All-rac* diet. The silage contained 39 mg/kg DM α -tocopherol and contributed substantially to the total intake of α -tocopherol (Table 1).

Table 1. Daily α -tocopherol intake (mg/d) in dairy cows fed daily supplements of no vitamin E (Control), RRR- α -tocopheryl acetate (RRR), all-rac- α -tocopheryl acetate and seaweed (SW) (n = 24)

Item	Control	RRR	<i>All-rac</i>	SW	SEM	P-value
Silage	570	574	571	562	21.0	0.9328
Concentrate	23.0 ^{ab}	22.7 ^{ab}	22.6 ^b	23.6 ^a	0.37	0.0209
Supplement	43.5 ^c	549 ^a	477 ^b	3.2 ^c	14.3	<0.0001
Total	636 ^c	1146 ^a	1070 ^b	591 ^c	16.6	<0.0001
RRR-isomer	624 ^{bc}	1086 ^a	649 ^b	591 ^c	16.5	<0.0001
Sum RSS, RRS, RSR	4 ^c	28 ^b	177 ^a	0 ^c	2.8	<0.0001
Sum 2S-isomers	8 ^c	32 ^b	244 ^a	0 ^c	4.2	<0.0001

SEM=Standard error of mean

^{abc} Means in same row with different superscripts are significantly different (p<0.05)

Compared to the control diet, RRR increased the total content of α -tocopherol in plasma by 32 % and in milk by 25%, while *All-rac* elevated the content in plasma with 13% but had no effect on milk (Table 2). When the differences in plasma and milk concentrations of α -tocopherol concentrations were adjusted to account for the differences in α -tocopherol intake, the concentration of α -

tocopherol per unit of α -tocopherol consumed was 1.09 and 1.12 times greater in plasma and milk, respectively, when cows were fed RRR than *All-rac*. Seaweed supplementation had no effect on plasma or milk content of α -tocopherol. *All-rac* resulted in higher concentration of the synthetic isomers (RSS, RRS, RSR and 2S) in both plasma and milk than the other treatments.

Table 2. Plasma and milk concentrations (mg/L) of α -tocopherol in dairy cow fed daily supplements of no vitamin E (Control), RRR- α -tocopheryl acetate (RRR), all-*rac*- α -tocopheryl acetate and seaweed (SW) (n = 24)

Item	Control	RRR	<i>All-rac</i>	SW	SEM	P-value
<i>α-Tocopherol in plasma</i>						
Total	9.99 ^c	13.17 ^a	11.27 ^b	10.13 ^c	0.632	<0.0001
RRR	9.83 ^b	12.82 ^a	9.69 ^b	10.06 ^b	0.578	<0.0001
Sum RSS, RRS, RSR	0.15 ^{bc}	0.33 ^b	1.47 ^a	0.07 ^c	0.076	<0.0001
Sum 2S	0.009 ^b	0.017 ^b	0.109 ^a	0.005 ^b	0.0051	<0.0001
<i>α-Tocopherol in milk</i>						
Total	1.20 ^{bc}	1.50 ^a	1.25 ^b	1.14 ^c	0.052	<0.0001
RRR	1.20 ^b	1.47 ^a	1.09 ^c	1.14 ^{bc}	0.050	<0.0001
Sum RSS, RRS, RSR	0.00 ^b	0.03 ^b	0.14 ^a	0.00 ^b	0.008	<0.0001
Sum 2S	0.001 ^c	0.005 ^{bc}	0.019 ^a	0.001 ^c	0.0011	<0.0001

SEM=Standard error of mean

^{abc} Means in same row with different superscripts are significantly different (p<0.05)

The *All-rac* diet had about 61 % of all isomers as RRR, whilst the RRR proportion in plasma and milk in cows on the *All-rac* diet was > 86 % (Table 3). On the other diets > 94 % of the isomers was the RRR-isomer in both diet and plasma and in milk.

Table 3. Relative proportions (%) of α -tocopherol stereoisomers in diet, plasma and milk in dairy cow fed daily supplements of no vitamin E (Control), RRR- α -tocopheryl acetate (RRR), all-*rac*- α -tocopheryl acetate and seaweed(SW) (n = 24)

Item	Control	RRR	<i>All-rac</i>	SW	SE	P-value
<i>Total diet</i>						
RRR	98.1 ^a	94.7 ^a	60.7 ^b	100.0 ^a	0.62	<0.0001
Sum RSS, RRS, RSR	0.5 ^b	1.8 ^b	16.6 ^a	0.0 ^b	0.26	<0.0001
Sum 2S	1.2 ^c	2.8 ^b	22.8 ^a	0.0 ^d	0.36	<0.0001
<i>Plasma</i>						
RRR	98.4 ^{ab}	97.4 ^b	86.6 ^c	99.2 ^a	0.45	<0.0001
Sum RSS, RRS, RSR	1.5 ^{bc}	2.5 ^b	12.4 ^a	0.7 ^c	0.42	<0.0001
Sum 2S	0.1 ^b	0.1 ^b	1.0 ^a	0.1 ^b	0.04	<0.0001
<i>Milk</i>						
RRR	99.4 ^a	97.9 ^a	87.4 ^b	99.6 ^a	0.60	<0.0001
Sum RSS, RRS, RSR	0.5 ^b	1.8 ^b	11.1 ^a	0.3 ^b	0.16	<0.0001
Sum 2S	0.1 ^c	0.3 ^b	1.5 ^a	0.1 ^c	0.08	<0.0001

SEM=Standard error of mean

^{abc} Means in same row with different superscripts are significantly different (p<0.05)

Discussion

The study confirms that natural α -tocopherol (RRR) is the pre-dominating form in blood and milk of dairy cows, irrespective of dietary source (Meglia et al 2006; Weiss et al. 2009). The results also shows that dairy cows in mid and late lactation, as periparturient cows (Meglia et al 2006), have a preferential uptake of RRR- α -tocopherol compared with the other stereoisomers of α -tocopherol.

Even in treatments with low α -tocopherol contribution from the supplements (SW and Control), the plasma concentration of α -tocopherol was high compared to other studies and 3 times higher than the level of 3 mg/L that is regarded as adequate (Beeckman et al. 2010, Lindqvist et al 2011). Even though the silage concentration of α -tocopherol (39 mg/kg DM) was not particularly high, but similar to levels observed in other studies (Beeckman et al. 2010, Lindqvist et al 2011), it is clearly adequate when the silage intake is as high as in the present study.

The financial support from TINE SA, the Foundation for Research Levy on Agricultural Products, the Agricultural Agreement Research Fund and the Research Council of Norway is acknowledged.

Suggestions to tackle the future challenges of organic animal husbandry

Do organically managed dairy cows in mid and late lactation on diets with high intake of grass-clover silage need extra supplement of vitamin E?

References

- Allen VG, Pond KR, Saker KE, Fontenot JP, Bagley PC, Ivy RL, Evans RR, Schmidt RE, Fike JH, Zhang X, Ayad JY, Brown CP, Miller MF, Montgomery JL, Mahan J & Wester DB (2001). Tasco: Influence of a brown seaweed on antioxidants in forages and livestock-A review. *Journal of Animal Science* 79, E21-E31.
- Beeckman A, Vicca J, Van Ranst G, Janssens GPJ & Fievez V (2010): Monitoring of vitamin E status of dry, early and mid-late lactating organic dairy cows fed conserved roughages during the indoor period and factors influencing forage vitamin E levels. *Journal of Animal Physiology and Animal Nutrition* 94, 736-746.
- Commission regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. *Official Journal L250*, 1-84.
- Govasmark E, Steen A, Strom T, Hansen S, Singh BR & Bernhoft A (2005): Status of selenium and vitamin E on Norwegian organic sheep and dairy cattle farms. *Acta Agriculturae Scandinavica Section A-Animal Science* 55, 40-46.
- Jensen SK & Nielsen KN (1996) Tocopherols, retinol, β -carotene and fatty acids in fat globule membrane and fat globule core in cows' milk. *Journal of Dairy Research* 63, 565-574
- Lindqvist H, Nadeau E, Waller KP, Jensen SK, Johansson B (2011): Effects of RRR- α -tocopheryl acetate supplementation during the transition period on vitamin status in blood and milk of organic dairy cows during lactation. *Livestock Science* 142, 155-163
- Meglia GE, Jensen SK, Lauridsen C & Waller KP (2006): alpha-tocopherol concentration and stereoisomer composition in plasma and milk from dairy cows fed natural or synthetic vitamin E around calving. *Journal of Dairy Research* 73, 227-234.
- Weiss WP, Hogan JS & Wyatt DJ (2009): Relative bioavailability of all-rac and RRR vitamin E based on neutrophil function and total α -tocopherol and isomer concentrations in periparturient dairy cows and their calves. *Journal of Dairy Science* 92, 720-731